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The structure of an integrin/talin complex reveals the basis of inside-out signal transduction

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(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 25 August 2009

Thank you for submitting your manuscript to the EMBO Journal. Your manuscript has now been seen by three referees and their comments to the authors are enclosed below. As you can see, the referees find the study interesting, well done and appropriate for the journal. They raise minor points that should not involve too much work to resolve. I would therefore like to invite you to submit a revised manuscript. When you send us your revision, please include a cover letter with an itemised list of all changes made, or your rebuttal, in response to comments from review. Please also make sure that relevant PDB codes are provided in the final version.

Thank you for submitting your interesting study to the EMBO Journal.

Yours sincerely,

Editor
The EMBO Journal

REFEREE REPORTS

Referee #1 (Remarks to the Author):

This is a well written paper describing a structure for the complex of integrin B1D cytoplasmic domain with the head domains of talin2. The interaction between these two partners is higher affinity than that of previously studied integrin-talin pairs allowing crystallization without the

artificial manipulations used in obtaining previous structures. The structure reveals the details of the interaction of the F3 domain of talin with the integrin tail, in which a lysine from the talin binds to an aspartate from the tail, competing for this D residue with a lysine from the integrin alpha subunit tail. This provides an explanation for the activation (breaking the D-K salt bridge between the two integrin tails). In addition the structure reveals a patch of conservedbasic residues on the talin F2 domain suitably placed to bind to phospholipids. Further experiments involving mutations of the candidate residues confirm the validity of these interpretations in the context of intact integrins and in binding assays with liposomes. Further contemplation of the structures shows that the talin binding would cause a rotation of the B TM segment away from its interactions with the alpha TM segment, thereby further separating the two integrin subunits.
This is a satisfying extension of our existing understanding of the activation of integrins by talin.
I have few criticisms of the manuscript, which seems to me to be ready to publish with minor revisions.

Minor Points

Despite the dictates of Microsoft Word, adverbs (well, evolutionarily etc.) do not need hyphens between them and the adjectives to which they apply - please fix.

LB is Luria broth, not lysogeny broth.

I am not convinced the movies add much.

Referee #2 (Remarks to the Author):

This is a clearly presented, technically excellent paper that provides novel mechanistic insights into integrin activation by talin. The authors find a relatively high affinity beta-integrin/talin pair, allowing them to obtain the first high-resolution crystal structure of a native talin-integrin complex. Using a previous model of the transmembrane region of a talin heterodimer, the authors construct a plausible model of how interaction of the talin N-terminal region with the membrane, as well as formation of a key salt bridge with the beta-integrin tail that competes with the alpha-integrin tail, leads to reorientation and separation of the integrin subunits needed for activation. Mutagenesis supports the key claims of the model, although the actual change in transmembrane orientation, if any, of the beta-integrin remains speculative.

The identification of a beta-integrin/talin pair with relatively high affinity was obviously convenient for structure determination, but the higher affinity observed with this integrin/talin pair is itself interesting. As the authors point out, the key residues thought to be involved in integrin activation are all conserved, so what is the structural basis for the higher affinity? Presumably the higher affinity is conferred by contacts between talin F2-F3 and the extreme C-terminus of the beta-integrin tail, but these interactions are not discussed in the manuscript.

The authors note that the higher affinity of this pair is consistent with the greater stress to which integrins are subjected in myotendinous junctions. In addition, an integrin with higher affinity for talin might be activated by a lower concentration of talin. Do cellular concentrations of these proteins fall in ranges where the difference in affinity might be important (i.e., where integrins with a higher affinity for talin might be selectively activated)?

A few minor points that should be addressed:
1. The 3.7 A distance between K327 and D759 is outside the range normally considered for a salt bridge. Are the B factors higher for these residues than average, i.e. is the error on the coordinates expected to be larger than the overall value given in the table? From the electron density map, it is not clear if the relevant atoms might actually be closer.
2. Were non-crystallographic symmetry con/restraints applied in the refinement? If so, the relevant statistics should be given. Also, could application of these restrains affect the distance between K327 and D759?

3. Table II has way too many significant figures in several places, for example the R values, bond length deviations, estimated phase angle error.
4. Figure 1 caption: Color coding of residues should be explained.

Referee #3 (Remarks to the Author):

The manuscript by Anthis et al. addresses the issue of the molecular basis of talin driven inside-out integrin activation using crystallography and in vitro functional analysis of mutant forms of the protein studied.
The results are interesting since the authors have used for the first time an authentic full length beta1 integrin cytoplasmic tail in complex with the C-terminal portion of talin. To achieve this goal the author used the cytoplasmic tail of the integrin isoform beta1D and talin 2, an integrin/talin pair that bind with higher affinity compared to other isoforms. This allowed to obtain a stable crystal and avoid the use of chimeric molecules possibly generating artefactual results. By comparing their results with previously published data, the authors achieved important new structural information mainly defining:
- a positively-charged patch on the talin F2 domain which binds to the membrane phospholipids and orient talin to disrupt the alpha/beta integrin transmembrane complex.
- a ionic interaction between the talin F3 domain and the membrane-proximal helix of the tail involved in disruption of an integrin / subunit interaction present in inactive integrin.
The functional relevance of these interaction has been convincingly confirmed by in vitro binding assay and expression in cellular system.

The results are critically analyzed and the conclusion are well supported by the data. Overall the data bring new information to the field.

1st Revision - authors' response 28 August 2009

We wish to resubmit a revised manuscript entitled “The structure of an integrin/talin complex reveals the basis of inside-out signal transduction” for your consideration as an article in The EMBO Journal. We thank the referees for their insightful comments and suggested revisions, which we have incorporated as described below.

Referee #1:

1. We have removed hyphens between adverbs and adjectives at all occurrences in the manuscript.

2. We have changed “lysogeny broth” to “Luria broth” in the Materials and Methods section.

3. Referee #1 wrote “I am not convinced the movies add much.” We respect the referee’s opinion on this matter, but we still believe that these movies help illustrate the activation model in a manner which the static figures in the manuscript are unable to. These are supplementary, after all, and since they will not contribute to the size of the printed paper, we would like to retain them.

Referee #2:

1. Referee #2 stated “the 3.7 *Å* distance between K327 and D759 is outside the range normally considered for a salt bridge.” We agree that this is a relatively large distance, but this is within the commonly used limit of 4.0 *Å*. We have updated the text to acknowledge this, and we have added references for the 4.0 *Å* salt bridge definition. Referee #2 asks whether the B values for these residues are higher than average and whether the interacting atoms are possibly closer. The

electron density for these atoms is well defined; The B values range from an average of 23 $Å^2$ for both C_β atoms, to an average of 31 $Å^2$ for the aspartate side chain carboxyl oxygen atoms, and 45 $Å^2$ for the lysine nitrogen atom, indicating that both residues are well structured (which is notable, since lysine side chains often display indistinct structures on protein surfaces). There could be some uncertainty in the position of the terminal lysine nitrogen atom, but the distance is similar in both integrin/talin dimers in the asymmetric unit (3.66 $Å$ vs. 3.70 $Å$). Although the distance is relatively large, an analysis by the program PISA predicts that D759 and K327 contribute favorably to the overall interaction, and the presence and importance of this salt bridge is backed up by the mutagenesis data presented in the manuscript. We have expanded the first paragraph of the section entitled "The talin F3 domain forms a key membrane-proximal salt bridge with the β integrin tail" to incorporate this more nuanced discussion of the structure.

2. Referee #2 asks if non-crystallographic symmetry (NCS) restraints were used in the refinement of the crystal structure and whether these could affect the D759/K327 distance. We used NCS restraints only in the initial stages of refinementónot in the latter stages. We have added a statement to this effect in the Materials and Methods section. Thus, the distances observed in each dimer in the asymmetric unit are expected to be an accurate reflection of the actual distance in that dimer and should not be affected by the application of NCS restraints early in the refinement process.

3. Referee #2 stated "Table II has way too many significant figures in several places, for example the R values, bond length deviations, estimated phase angle error." We agree that some of these values have more significant figures than necessary. We have reduced the number of significant figures in R_{work}, R_{free}, B value, and estimated phase error. We have also now expressed the R values in %, which is more conventional.

4. We have added an explanation of the color coding of residues in Figure 1.

5. Referee #2 asks "what is the structural basis for the higher [β1D/talin2] affinity?" We agree that this is a very interesting and important question, but one that is beyond the scope of the current manuscript; any comments at this stage would be speculation. This is an area of ongoing study for us, and we hope to submit a manuscript on this subject in the future when we have completed further experiments. We have extended the last paragraph of the section entitled "ìThe structure of the talin2/ 1D complex" to emphasize that we do not address this question in the current manuscript.

6. Referee #2 also asks "Do cellular concentrations of these proteins fall in ranges where the difference in affinity might be important (i.e., where integrins with a higher affinity for talin might be selectively activated)?" This is also an interesting question, but a particularly complicated one (that is also beyond the scope of the current manuscript). The overall concentrations of talin and integrins in the cell alone would not supply a clear answer since one has to consider effective local concentrations and 2D diffusion along the membrane surfaceófactors which complicate things greatly. We do have some preliminary data that indicate that the differences in affinity for these integrin tails influences the adhesion of cells to extracellular matrix molecules, but this is an area that requires further study.

Referee #3 did not raise any issues or suggest any revisions.

Thank you for your time and consideration.

